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Original Article

Periodontitis Therapy with Periodontal-derived Stem Cells (PDSCs) for Dental Tissue Regeneration

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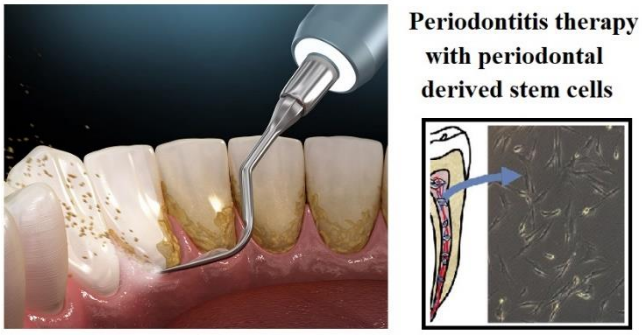
ABSTRACT

Periodontitis is a severe gum infection that can lead to tooth loss and other serious health complications. Periodontal ligament stem cells (PDLSCs), which reside in the perivascular space of the periodontium, possess characteristics of mesenchymal stem cells and are a promising tool for periodontal regeneration. The objective of this study was to assess the regenerative capacity of PDLSCs and osteoblasts differentiated from PDLSC among periodontal patients. This research was conducted from February 17, 2019 to December 11, 2021. A sample of 30 patients with periodontal disease with bone disease (losing the study) was identified from the pilot study sample by means of conventional sampling. Randomly assigned to two sets of eyelids, 15 subjects. Group A contained PDLSCs patients, while group B patients were a control group. After documenting surface markers measured upon cytometry, SCs were differentiated after their extraction as explained by the previous researchers. Defects are detailed with PDLSCs, blanks and fonts. After 4 weeks, tissue documentations containing air, alizarin and BMP4 staining, PGLAP gene, etc. RT-PCR preparation was used. The results of the current study for CD-90, an autumnal stem cell marker, were avian strains in both groups. Alizarin red staining was performed to show mineral substances. RT-PCR confirmed the differentiation of chondrocytes and bone. The study concluded the posterior cells of PDLSCs (other cells) to function in soft and hard tissues.

GRAPHICAL ABSTRACT



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Introduction

Periodontitis is an acute inflammatory disease of both the periodontal ligament and the alveolar bone, clinically presenting symptoms such as loss of PDL with periodontitis eventually leading to periodontal bone loss [1–4]. Stem cell therapy, which works on the basis of regeneration and repair of damaged tissues, is a promising new advance among dental procedures [5–8]. Factors that play a major role in the success of stem cell regeneration therapy are tissue regeneration and factors such as cells, scaffolds, and signaling molecules [9–13]. Clinically, scaffolds, which usually do not have the inducing capacity, require the use of growth factors to promote the regeneration process, but stem cells can act differently [14].

Stem cells are non-immune with high regeneration potential and can be easily transplanted from different parts of the body such as bone marrow, skin, cornea, periodontal ligament (PDL), etc. [15,16]. PDL is tissue located between the part of the alveolar bone and the cementum of the tooth. Fibroblasts and progenitor cells that can further differentiate into stem cells and chondrocytes, their formation in the stromal cell population has recently been demonstrated, more recently [17–20]. Teeth with tissue homeostasis preserved affected tissues. It has improved the gums due to the presence of these cells in the progenitors [21–23]. Another tissue propagated into regressive bone marrow mesenchymal stem cells (BM-MSCs) seen can differentiate into bone, chondrocytes, and other soft tissues [24–26].

The aim of this study is the cells belonging to the background of the periodontal ligament stem cells, their differentiation, and their ability to regenerate.

Materials and Methods

Before the investigation, the participants were informed of the objectives of this study. Written consents were obtained from them prior to conduct the study. Ethical approval had been obtained from the Ethics Committee of Medical University. This research was conducted from February 17, 2019 to December 11, 2021. A sample of 30 patients was appropriately selected for this experimental study. Group A received PDLSCs treatment, while group B remained as a control group. Stem cells were extracted in the following way:

Isolation and culture of PDLSCs

The gums were separated with a scraper from the root surface of the lower central incisors. Sections were divided into many sections. These sections were washed in DMEM culture medium. Sections were kept in type 1 collagenase buffer and further enzymatically digested for 1 h at 37 °C to isolate PDLSCs from adjacent dental tissues. A mixture of cell suspensions of low-glucose DMEM, 10% FBS, L-glutamine, 100 units of penicillin, 100 mg/ml streptomycin, 5 mg/ml amphotericin B, and 0.05% EDTA, were used to analyze the organ immunological parameters.

Stem cell evaluation

In the process, cells were treated and counted with trypsin-EDTA. They were incubated and centrifuged at 1000 rpm for 5 min. 3% FBS was added to the culture medium, at room temperature for half an hour. After centrifuging the mixture at 1000 rpm for 5 min, anti-CD166, anti-CD105, anti-CD45, anti-CD34, anti-add-CD73, anti-CD44, anti-CD90 (FITC) and anti-CD146 were observed. This mixture was kept at 4°C for 45 min.

Differentiation of PDLSCs

Isolated PDLSCs were placed in DMEM buffer containing 10% FBS, 10 mM glycerol phosphate, 10–7 M dexamethasone and 50 mg/ml ascorbic acid. Alizarin red staining technique was used to analyze cell differentiation.

Alizarin red staining

For this culture medium, it was washed with Tyrode's solution and PBS before being fixed in 4% paraformaldehyde. Cells were subsequently stained with 40 mM alizarin red for 10 min. After staining they were rinsed with water and Solution PBS.

RT-PCR

This procedural technique was performed to analyze and confirm the differentiation of PDLSCs into osteoblasts. Expression of BMP4, Cbfa1, and

BGLAP have been documented as osteoclast-specific genes. In the detection of PDLSCs, the DLX3 gene plays an important role. Glorify him in documenting his existence. The matters were related in the following way: Fw (BMP4): 5' GCC GGG GAA GAG GAG GAG 3', Rev (BMP4): 5' CAA TAT GGT CAA AAC ATT TGC 3', Fw (Cbfa1): 5' ATG CTT CAT TCG CCT CAC AAA 3', Rev (Cbfa1): 5' AAG CTT TGC TGC TGA CAC GGT GTC 3', Fw (BGLAP): 5' AGC CAC CGA GAC ACC ATG AGA 3', Rev (BGLAP): 5' TTT TCA GAT TCC TCT TCT GGA G 3', Fw (Dlx3): 5'CTA CCG GCA ATA CGG GGC GT 3', Rev (Dlx3)**:** 5'AGT GGA GTG GGA AGA GGT GTC CCA 3'. The DNA preparation process was carried out in a thermocycler. Polymerase chain reaction was carried out using 25 µl of reaction solution with 3 µl of mRNA, 8.0 µl of water, 1 µl of RNase enzyme, 4 µl of inverted primer, 2 µl of reaction, 25 µl of dNTP mixture and 5 µl buffer.

To Rejuvenate Stem Cell Regeneration Activity. Then, PDLSCs and primary osteoblasts cell suspensions were placed on the membrane-bound group A patients.

Histological extraction and investigation

The extracted bones were immersed in 10% formalin. After fixation and placement in paraffin, 5-µm sections were cut. These were stained with H&E, and then examined. In histological evaluation, bone regeneration was performed dynamically, while the regenerated bone was attached to the bone adjacent to it without retirement tissue.

Immunohistochemistry of cultured cells

Cultured PDLSCs sub-diluted with anti-STRO-1 and anti-CD146 were incubated overnight at 4°C, rinsed with PBS, and incubated with a second antibody with Phycoerythrin (PE) at room temperature for 45 min. Combat slides have been installed.

Statistical analysis

The significance has been evaluated. A P-value less than 0.05 was considered statistically significant.

Results

Among the selected samples aged 40–50 years old, 56% of the females belonged to group A, while 60% were to group B (Table 1 and Figure 1).

Table 1: Gender distribution

	Gender	
Sub	Group A	Group B
Male (in %)	44	40
Female (in %)	56	60

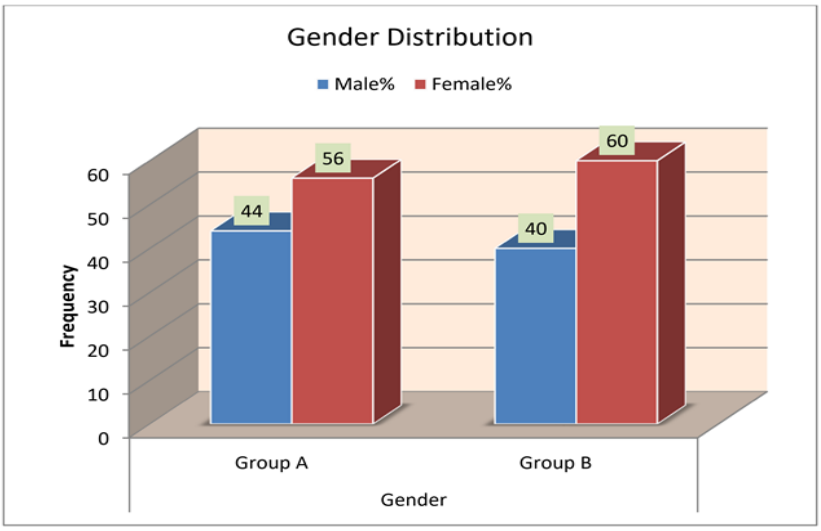

Figure 1: Gender distribution

Flow cytometry

For the group, cells were assessed for the expression of cell surface markers resulted in a successful outcome for PDLSCs. Background cells for the cell markers CD90, CD105, CD73, and CD166, specifically categorized by stem cells;

however, cells were negative for CD45 and CD34, and CD44 surface markers. More than half (59.49%) of the PDLSCs expressed the CD90 surface marker. The characteristic but non-referential CD146 marker was also found in the gingiva (Table 2 and Figures 2 and 3).

Table 2. Surface markers for PDSCs

Gene Marker	Expressed Percentage
CD44	96.9%
CD90	59.4 %

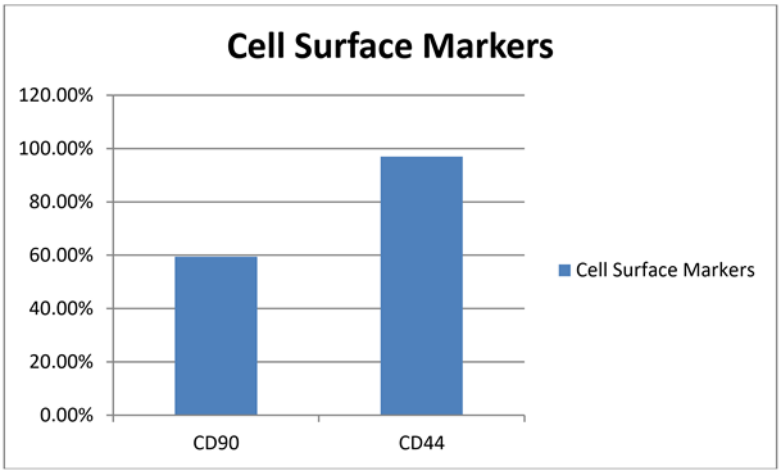

Figure 2: Cell surface markers

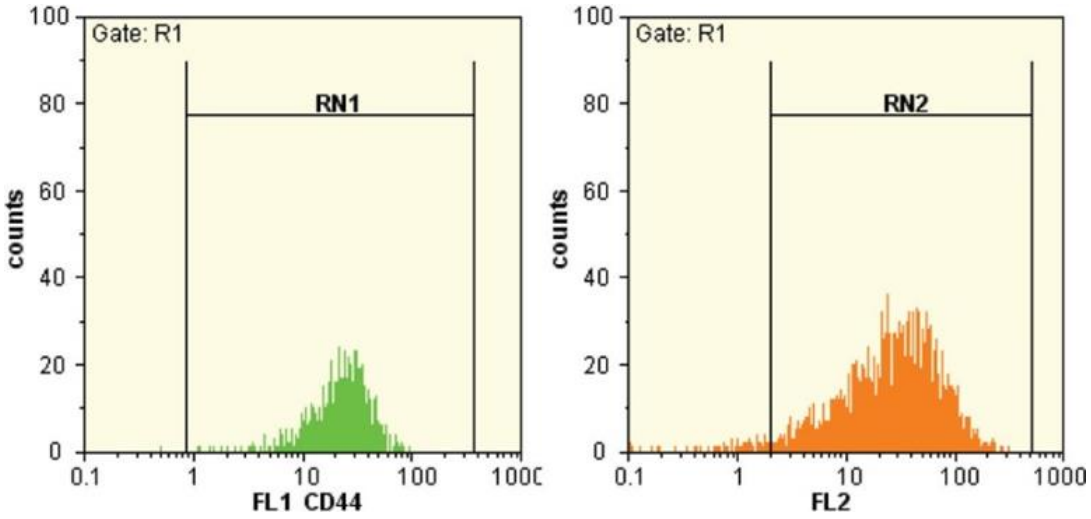

Figure 3: Surface markers by Flow cytometry

Stem cells differentiate into osteoblasts

When differential cytokines were added, stem cells differentiated into osteoblasts. Well calcium excretion was shown by alizarin red staining which indicated calcium deposition in red-orange. The progenitor cells of PDLSCs differentiated well into osteoblasts and chondrocytes. Stem cell nuclei were stained with DAPI (blue) and demonstrated the osteogenic activity of the cells (Figure 4).

Figure 4: Osteoblastic activity as shown by alizarin staining

RT-PCR

Bone cell-specific gene expression measurement was performed to confirm osteogenic differentiation. 1.5% agarose gel electrophoresis and ethidium bromide staining were applied. Quantification was performed by fermented DNA tags. DLX3 increases during gingival formation in the values of the control group.

Morphological assessment

In group A, the studied variables, consisting of inflammation 5.1.3 bone, had significant changes ($p < 0.0001$). After four weeks, there were no significant differences between groups in which bone was regenerated by PDLSCs prior osteoclasts (Figure 5).

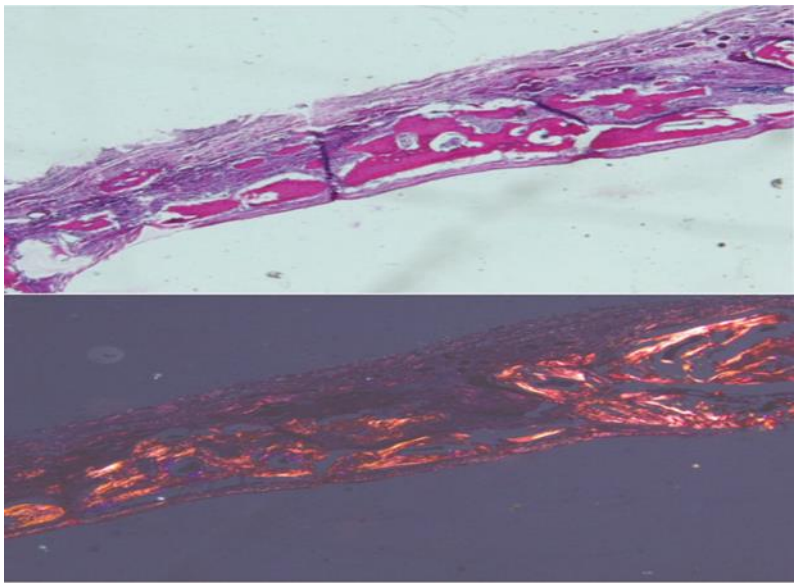

Figure 5: Microscopic view PDSCs

The resulting equivalent of comparing inflammation and new bone formation in the experimental group had a significant difference. There was a significant difference between groups and assignment to PDLSCs ($p = 0.018$) adjusting to control ($p = 0.001$). Regarding the newly formed cause about the cause of the emergency problem.

Discussion

The reasons that appeared in the freezing of cells led to the tooth freezing. These stem cells consist of stem cells, stem cells of cells that not only have the potential to differentiate into bone-forming cell as well as fat cells and osteoblasts [27,28]. The presence of stem cells in PDL was examined by cytological analysis.

For the current study, stem cells were isolated from PDL. Expression of specific surface markers such as CD90, CD105, CD166, and CD73 confirms isolation from PDL. Expression of CD44 and CD146 also confirms the sequestration of PDLs. PDLSC is placed in a cryogenic differentiation medium over cytokines.

Verifying osteoblastic differentiation, calcium was traced with alizarin red staining. Red colored appearance after staining to osteogenesis. To further confirm this picture, osteoclast-specific gene expression was evaluated. RT-PCR technology was used for this purpose. The formation of the Cbf1 protein is essential for the differentiation of cells into osteoblasts. BGLAP or Osteocalcin was another gene that was characterized. It is the most specific brand of osteoblasts, and the work on the bulk of this gene, plays an essential role. Furthermore, it has been indicated that PDLSCs have the ability to express osteoblasts and cementum cell markers, forming dentin and osteoblasts in vivo for the previous studies. It is necessary for the differentiation and skeletal formation of osteoclasts. It is also soft. As a consequence of the factors, DLX3 gene expression can also be considered as a marker of cell differentiation.

In the process of tooth formation, DLX3 is initially expressed in the dental epithelium, and then in the dental stromal epithelial cells (DE-DM). The transcription factors in tooth root development are DLX2 and DLX3. They are necessary for the growth of the periodontal ligament.

Conclusion

The results of this study showed that stem cells and osteoblasts proliferated and differentiated by transferring them to the defect. The reason for the presence of cell restarts, cell reappearance of PDLSCs changed into progenitor cells, differentiation into osteoblasts, and finally osteoblasts can be attributed to remodeling. The second role of environmental induction in the reconstruction process, has little effect on PDLSCs. The cells appeared to have developed from the control group. The results of the previous studies guarantee this. From sending military assistance to rebuild enemies in the control group, it can be the joint resignation badge of the military cells of the military cells. In general, it can be concluded that PDLSCs can obtain osteoblast differentiation potential. The results of the current study indicate that the regenerative potential of this type of stem cell is more than autologous bone healing.

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Authors' contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

There are no conflicts of interest in this study.

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